

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of : Hiroshi NAGAI, Masaaki YASUE and Mari
YAMAMOTO
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For : Functional Beverage and Composition
Examiner : Melissa Perreira
Art Unit : 1618

Honorable Commissioner of Patents and Trademarks
P.O. Box 1450
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DECLARATION UNDER 37 CFR §1.132

I. Mari YAMAMOTO, a citizen of Japan, do hereby declare the following:

2. I received a Bachelor of Science degree in agricultural chemistry from the Department of Food Nutrient Chemistry in the Faculty of Horticulture at Chiba University, Chiba, Japan on March in 1984; a Master of Science degree in agricultural chemistry from the Faculty of Horticulture at Chiba University, Chiba, Japan on March in 1986; and a Doctor of Philosophy degree in Agriculture on September, 1992 from Kyushu University for a thesis entitled " Study on Regulation of Immunoglobulin Production by Food Components".

3. I am an employee of the National Institute of Vegetable and Tea Science, National Agriculture and Food Research Organization, Japan, the assignee of the above-identified United States patent application and, since April, 2004, have been engaged in research and development work relating to functional study of tea, especially anti-allergic and anti-obesity action of *O*-methylated catechin in green tea (cv. Benifuuiki), changes in chemical components of tea and other related products of the National Institute of Vegetable and Tea Science, National Agriculture and Food Research Organization, Japan.

4. I am one of the joint inventors in the above-identified pending United States patent application.

5. The attached experimental results shows a beverage derived from green tea containing *O*-methylated catechins having superior effects of reducing the triglyceride level, weight, fat content surrounding testes and liver triglyceride level compared to a green tea beverage containing catechins, but not containing *O*-methylated catechins, as well as compared to barley tea, which does not contain significant amounts of any forms of catechin.

6. Specifically, the results in Table 1 of the attachment indicate the total content of *O*-methylated catechins and total content of catechins in the beverages obtained from the extraction

of tea leaves of Benifuuki, Yabukita and barley. From this, it can be seen that beverages obtained from the extraction of tea leaves of Benifuuki contain significant amount of catechins, including *O*-methylated catechins. In contrast, the beverage obtained from the extraction of tea leaves of Yabukita contain catechins, but not *O*-methylated catechins, and the barley beverage does not contain detectable quantities of any forms of catechin.

7. Table 2 of the attachment shows an effect of reducing triglyceride levels of Test Beverages 1 and 3 of experiment 1. From the results shown in Table 2, it can be seen that the Test Beverage 1 (which includes a solution extracted from tea leaves of Benifuuki) was able to reduce triglyceride levels considerably when compared to the Test Beverage 3 (which includes a solution extracted from barley tea).

8. Furthermore, Table 3 of the attachment shows components of beverages obtained from the extraction of tea leaves of Benifuuki, Yabukita and barley. From the results shown in Table 3, it can be seen that the Benifuuki green tea of the present invention contains *O*-methylated catechins in an amount of 6.8 mg per 100 ml of the beverage, whereas Yabukita green tea and barley tea do not contain *O*-methylated catechins.

9. Table 4 shows an average change in triglyceride levels of a group of test subjects as a result of intake of the beverages listed in Table 3. From the results shown in Table 4, it was demonstrated that the average triglyceride level in the group of test subjects was significantly lowered after six weeks of consuming the Benifuuki green tea, whereas triglyceride levels in test subjects were not significantly lowered after six weeks of consuming the Yabukita green tea or barley tea.

10. These results showed that the beverages containing *O*-methylated catechins have remarkable and unobvious effects of reducing triglyceride levels compared to beverages not containing *O*-methylated catechin. In particular, neither barley tea without significant amounts of catechin, nor the green tea beverage obtained from a strain of tea containing significant amounts of catechins, but no methyl catechins, had a significant effect on triglyceride levels. In contrast, the beverage made from the strain of tea containing methyl catechins significantly reduced triglyceride levels.

11. I hereby declare that all statements made herein are to my own knowledge true and that all statements made on information and assumptions are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Jul. 29, 2010
Date


Mari YAMAMOTO

ATTACHMENT

1. Experiment 1

Measurement of catechin content of tea leaves

(a) Test Beverage 1

"Benifuuki" tea leaves were extracted at 90°C using purified water in an amount 30 times that of the tea leaves to produce a liquid extract, then a sodium bicarbonate water conditioner and vitamin C were added and mixed. The mixture was sterilized and filled into a container (a 250 ml paper pack in this example) under nitrogen and sealed to prepare Test Beverage 1.

(b) Test Beverage 2

"Yabukita" tea leaves were measured up by purified water such that the contents of the components other than the *O*-methylated catechin were the same as those of Test Beverage 1, then a sodium bicarbonate water conditioner and vitamin C were added and mixed. The mixture liquid was sterilized and filled into a container under nitrogen and sealed to prepare Test Beverage 2.

(c) Test Beverage 3

"Barley tea" was measured up by purified water such that the contents of the components other than the *O*-methylated catechin were the same as those of Test Beverage 1, then a sodium bicarbonate water conditioner and vitamin C were added and mixed. The mixture liquid was sterilized and filled into a container under nitrogen and sealed to prepare Test Beverage 3.

The content of *O*-methylated catechins within Test Beverages 1 to 3 are shown in Table 1.

TABLE 1

| (mg/100ml) | Test Beverage 1 | Test Beverage 2 | Test Beverage 3 |
|---|-----------------|-----------------|-----------------|
| Total Content of <i>O</i> -methylated Catechins | 8.1 | 0 | 0 |
| Total Content of Catechins | 104.8 | 109.2 | 0 |

2. Experiment 1

Study of the Triglyceride-Lowering Effect

Ten healthy adults, whose fasting triglyceride levels were 100 to 350 mg/dL, were divided into two groups of five persons (each group having four men and one woman), and were directed to drink Test Beverage 1 or Test Beverage 3 of Comparative Example 2 for six weeks. Each beverage was contained in a 250 mL bottle, and the subjects were directed to drink two bottles per day (500 ml/day). The triglyceride levels (average \pm SD value) were determined after fasting overnight at the test initiation and at the last drinking. The results are shown in Table 2. These results demonstrate that the intake of Test Beverage 3 containing no *O*-methylated catechin had no effect on serum triglyceride levels, whereas Test Beverage 1 containing *O*-methylated catechin significantly lowered triglyceride levels.

TABLE 2±

| | Week 0 | Week 6 |
|------------------------------|--------------|--------------|
| Test Beverage 1 (Benifuuki) | 174 \pm 82 | 125 \pm 62 |
| Test Beverage 3 (Barley tea) | 174 \pm 31 | 181 \pm 42 |

*p < 0.05 compared to week 0

3. Experiment 3

Subjects over the age of 18 years were divided into three test groups, and were directed to drink “Yabukita” green tea and barley tea twice daily, during breakfast and lunch, or within 30 minutes after these meals, for six weeks. The daily intake of test beverages was 500 ml/day (250 ml x 2 bottles).

The analyzed data of components of each test beverage are shown in Table 3. “Benifuuki” green tea contained 17 mg of *O*-methylated catechin in a 250 ml bottle. As examples for comparison, “Yabukita” green tea containing tea catechins but not containing *O*-methylated catechins, and barley tea having no detectable level of catechins were used. “Yabukita” green tea was produced such that the analyzed amount of tannins and caffeine, which was approximately equal to the total content of tea catechin, was the same as those of “Benifuuki” green tea.

TABLE 3

| | “Benifuuki” green tea | “Yabukita” green tea | Barley tea |
|------------------------------------|-----------------------|----------------------|------------|
| Energy (Kcal) | 0 | 0 | 0 |
| Protein (g) | 0 | 0 | 0 |
| Fat (g) | 0 | 0 | 0 |
| Carbohydrate (g) | 0 | 0 | 0 |
| Tannin (mg) | 120 | 118 | 0 |
| Caffeine (mg) | 22 | 22 | 0 |
| <i>O</i> -methylated Catechin (mg) | 6.8 | 0.0 | 0.0 |

Each data indicates a value per 100 ml of the beverage.

The blood of the test subjects was collected after six weeks of consuming the teas, and the triglyceride level in the blood was measured. The results are shown in Table 4.

From the results shown in Table 4, it can be seen that “Benifuuki” green tea significantly lowered triglyceride levels in the group of test subjects who consumed the “Benifuuki” green tea. No other beverage demonstrated such results.

Table 4

| Item | Standard value | Test beverages | Before the initial drinking | 6 weeks after the initial drinking |
|----------------------|----------------|-----------------------|-----------------------------|------------------------------------|
| Triglyceride (mg/dl) | 35-149 | “Benifuuki” green tea | 90±59 | 75±42 # |
| | | “Yabukita” green tea | 79±26 | 83±40 |
| | | Barley tea | 110±85 | 95±59 |

The values indicate average value±standard deviation

$p < 0.05$ compared to week zero

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